

# Study on the reproductive capacity of bulls of the autochthonous Rhodope Shorthorn cattle breed

## Проучване на репродуктивната способност на бици от автохтонната порода Родопско късорого говедо

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### Abstract

The sperm production of bulls from the autochthonous Rhodope Shorthorn cattle breed was studied. The breed is among the smallest in Europe, the average weight of the cows ranging from 200 to 250 kg, and of the bulls from 330 to 370 kg. It was found that during the first 6 months from the start of exploitation, at the age of the bulls from 18 to 24 months, AI bulls had high reproductive capacity. The ejaculate volume was  $1.74 \pm 0.09$  ml in average (LS), the percentage of motile spermatozoa was  $74.3 \pm 3.48\%$  and the concentration  $1268 \pm 13.1 \times 10^6/\text{ml}$ . It was established that the bull had a significant impact on the reproductive performance, but the individual differences in the main characteristics were not high – motility 71.8-77.0%, concentration –  $1222-1324 \times 10^6/\text{ml}$ . The season also had a significant effect on the percentage of motile spermatozoa. Within the period from January to June, the highest reproductive capacity of the bulls was observed from February to May and the lowest in June.

**Keywords:** autochthonous breeds, cattle, reproduction, Rhodope Shorthorn cattle, semen

### Резюме

Проучена е спермопродукцията на бици от автохтонната българска порода Родопско късорого говедо. Породата е сред най-дребните в Европа, като средното тегло на кравите е 200-250 kg, а на биците- 330-370 kg. Установено е, че в първите 6 месеца от началото на експлоатация, при възраст на биците 18 – 24 месеца, разплодниците са имали висока репродуктивна способност. Обемът на еякулата е средно (LS)  $1.74 \pm 0.09$  ml, подвижността на спермата е  $74.3 \pm 3.48\%$  и концентрация  $1268 \pm 13.1 \times 10^6/\text{ml}$ . Установено достоверно влияние на разплодника ( $P < 0.001$ ) върху репродуктивната способност, но индивидуалните различия по основните признаци не са високи – по подвижността 71.8-77.0%, по концентрация –  $1222-1324 \times 10^6/\text{ml}$ . Върху подвижността на сперматозоидите достоверно е влиянието и на сезона на получаване на спермата. През периода януари – юни, най-висока е била

репродуктивната способност на биците през месеците от февруари до май, а най- лоша през месец юни.

**Ключови думи:** автохтонни породи, говеда, репродукция, Родопско късорого говедо, сперма

## Introduction

The Rhodope Shorthorn cattle (RShC) is a Bulgarian autochthonous breed. It is a primitive form of *Bos taurus brachyceros* and according to Academician Hlebarov (1940-1941) it is closer to “the fossil domestic cattle from the Neolithic Age (the peat cattle) than the cultural alpine *brachyceros* types. It represents last remnants of the prehistoric European cattle, along with the types described by Adametz which include the Albanian south, Montenegrin and Illyrian cattle, with which it is in the closest genetic relationship”.

The cattle from the breed RShC are among the smallest in Europe. For cows, the average height at the withers is 104.2 cm, the oblique length - 125.0 cm, the chest girth - 146.4 cm (Nikolov, 2012). The average weight of cows is 200-250 kg, and of bulls - 330-370 kg. The milk-yield is low, and for lactation 800-1200 liters of milk are obtained with 4.5% fat (Tsonev and Vasilev, 1962).

The breed is bred primitively in the poorest mountainous and hilly parts of the Rhodope Mountains. In 2012, 695 cows and 41 bulls were under selection control (Malinova and Nikolov, 2012). According to the classification of FAO (FAO, 2007), the breed is endangered, and because of this special attention is paid for its conservation.

In “The Global Strategy for the Management of Farm Animal Genetic Resources” (FAO, 1999) a radical change in the concept of global biodiversity conservation of domestic animals is recommended - from focusing on *ex-situ* methods, to concentration of efforts to protect and develop the autochthonous breeds within the relevant natural areas (*in situ*). However, the preservation of the genetic diversity by keeping live animals outside their original or inside their usual production environment, may not always guarantee that the genetic diversity of the breed is maintained. Because of this, it is recommended (FAO, 2012 ) *in vivo* that the conservation should be complemented by cryoconservation of germplasm.

Semen from only three bulls from the RShC breed is preserved in the National Genetic Bank for farm animals in Bulgaria, and no studies of the semen production of the bulls have been made. Establishment of the main characteristics of semen production of bulls from this breed is the aim of the present study.

## Material and methods

In the survey are included three bulls from the Rhodope Shorthorn cattle breed reared in an Artificial insemination station in Sofia, owned by the state Executive Agency on Selection and Reproduction in Animal Breeding. Before entering the station, the bulls are assessed according to exterior, physiological and health status. Animals enter the station if having a pedigree certificate issued by the breeding organization.

The daily and diet regimes are followed strictly. Certain hours are intended for provision of compounded fodder, tethering of bulls, toilet and preputial lavage of every stud bull, then mounting in the days for obtaining semen, if it is not such a day, half of the daily ration of roughage is provided, after that, in nice weather, the bulls

are permitted to the boxes outside for a walk. Then follows cleaning of the site in front of the collection area, indoor boxes and food trail. Lunch break for the bulls. Next comes the setting of the other half of the portion of compounded fodder, cleaning the outside yards, setting of the second half of the roughage for the day, cleaning the food trails, the indoor boxes, covering of the beds with straw. The compounded fodder is made in a fodder factory according to a special recipe for stud bulls.

The bulls that are subject of the study are aged from 18 to 24 months, and have entered the station at the end of October 2012. In November and December the bulls are accustomed to obtaining semen and from January 2013 are included in the production mode.

The study of the reproductive capacity of bulls covers a six-month period (from January to June 2013). For the specified period have been collected and examined 120 ejaculates.

Semen is obtained by artificial vagina twice a week and as a dummy another bull is used. Before proceeding to obtaining, a preputial lavage in disinfectant solutions is made.

The volume of the ejaculate is assessed with the help of graduated pipettes with accuracy up to 0,1 ml. The progressive semen motility (%) is determined on a microscope slide with a glass coverslip, preheated to a temperature of 37-38 ° C, with consideration to the nature of the sperm movements, respectively progressively motile spermatozoa. Samples are evaluated immediately upon receipt of ejaculate under a light microscope at a magnification of x160. The semen concentration is calculated with a Burkner's counting chamber. The total concentration is obtained by multiplying the concentration in 1ml by the ejaculate volume (ml). The same operator that measures the volume and concentration assesses the semen motility.

The data have been processed using multifactor dispersion analysis, using two linear models of the following statistical type:  $Y_{ijk} = \mu + SY_i + MY_{ij} + I_k + e_{ijk}$  (Model 1);  $Y_{ik} = \mu + MY_j + I_k + e_{ik}$  (Model 2), where :  $Y_{ijk}$  - surveillance vector;  $\mu$  - overall average constant;  $SY_i$ ,  $MY_j$ ,  $I_k$  are fixed effects respectively of the season of obtaining the semen ( $i = 3$ ), the month of obtaining the semen ( $j = 6$ ), the individual ( $k = 3$ );  $MY_{ij}$  - random effect of the  $j$ -th month in the  $i$ -th season;  $e_{ijk}$  - residual variance.

## Results and discussion

The average values (LS) of the parameters that characterize the quality of the semen of bulls of the Rhodope Shorthorn cattle breed are presented in Table. 1.

Table.1. Seminal characteristics of RSh bulls.

Semen traits	N	LS	±SE	CV	Min	Max
Volume, ml	120	1,742	0,089	35,52	0.5	3.0
Motility, %	120	74,28	0,415	3,48	70	80
Concentration, $1.10^6$ /ml	120	1268	13,11	7,16	970	1530
Total concentration, $1.10^6$	120	2186	114,5	36,08	565	4340

The average ejaculate volume for the period studied is 1,742 ( $\pm 0,089$ ) ml, as individual variation is in a wide range (CV = 35,52%). In a study of the reproduction

capability of bulls of the Holstein and Brown Swiss cattle breeds we found (Nikolov, 1997) that the average ejaculate volume for the first 18 months, is respectively 5.34 and 5.55 ml. The main reason for the low ejaculate volume of bulls of the RShC breed is probably the significantly lower weight. It is not excluded that with age the ejaculate volume may increase. The dynamics of the index in the period studied gives us a reason for this. From Fig. 1 it is apparent that the ejaculate volume is increased in the period from January to June, from 1,51 ml to 2,07 ml, although the change is not linear. In the above mentioned study of Nikolov (1997), the difference in the ejaculate volume of bulls of the Holstein breed for the first 18 months and the whole period of economic use is 10.7% and of the Brown Swiss bulls - only 5.4%.

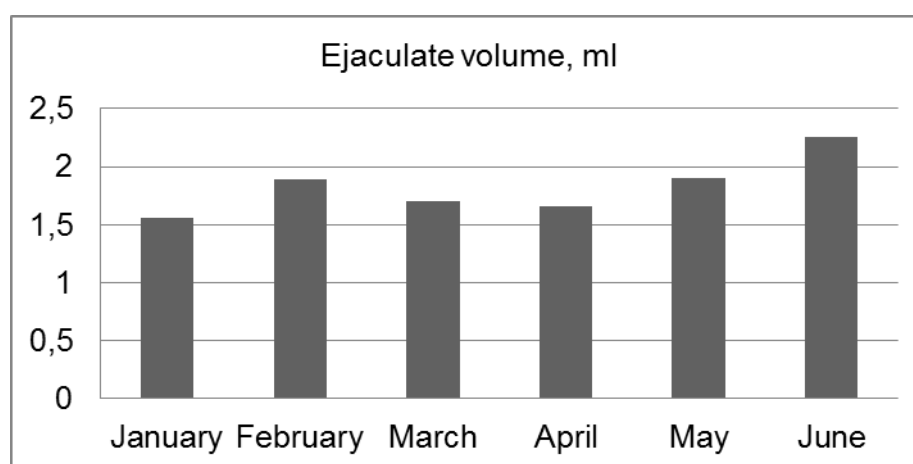


Fig.1. Monthly dynamics of ejaculate volume of RSh bulls

Table.2. Influence of the individual, month and season of the obtaining the semen on its quality

Semen traits	Individual	F-criteria and level of confidence			
		Model 1	Month in the season	Model 2	Month
		Season		Individual	
Volume	8.023***	2.065	0.895	8.023***	1.253
Motility, %	36.779***	5.558*	1.164	36.779***	3.154**
Concentration	11.218***	1.075	0.784	11.218***	0.806
Total concentration	4.565*	1.379	1.011	4.565*	1.155

\*\*\*P<0,001

Individuals have confidently ( $P < 0.001$ ) differed in ejaculate volume, while the season and the month of obtaining the semen have not significantly affected it (Table 2). The average ejaculate volume in individual bulls ranges from  $1.511 \pm 0.118$  to  $2.076 \pm 0.129$ , and during the certain months - from  $1.635 \pm 0.133$  in January to  $2.123 \pm 0.207$  in June.

The semen of bulls of the RShC breed has high concentration and the spermatozoa are with high motility. The concentration of spermatozoa per milliliter semen averaged to  $1268 \pm 13,11 \times 10^6/\text{ml}$  as the individual variation between ejaculates is 7.16%. The average percentage of motile spermatozoa is  $74,28 \pm 0,415$  and the

individual variation is low ( $CV = 3,48\%$ ). The total concentration of spermatozoa in the ejaculate averaged to  $2186 \pm 114,5 \times 10^6$ .

Individuals have significantly differed in the motility of spermatozoa and semen concentration. Spermatozoa motility for different bulls ranges from  $71.7 \pm 0.51$  to  $76.9 \pm 0.48\%$ , and the concentration from  $1222 \pm 17.9 \times 10^6/\text{ml}$  to  $1324 \pm 16.6 \times 10^6/\text{ml}$ , moreover, the bull with the highest semen concentration has also the highest motility of spermatozoa and vice versa.

In a study of the semen production of bulls of the Holstein and Brown Swiss cattle breeds, under the same conditions of feeding and breeding, Nikolov (2007) has found that during the first 18 months of exploitation, the semen concentration was respectively  $1155 \pm 19.5 \times 10^6/\text{ml}$  and  $1054 \pm 16.3 \times 10^6/\text{ml}$ , and motility respectively  $70.6 \pm 0.28\%$  and  $69.8 \pm 0.68\%$ , as with age both indicators were reduced. The data obtained show that the autochthonous breed of RShC has a better semen quality than that of the commercial.

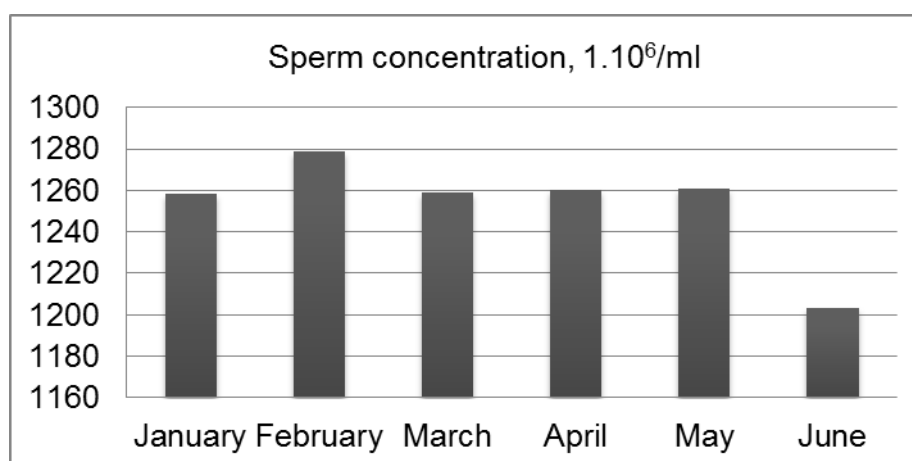


Fig.2. Monthly dynamics in sperm concentration of RSh bulls

In a study of the seasonal changes in the ejaculate characteristics Igboeli and Rakha (1971) have found that the ejaculate volume, the morphologically normal spermatozoa and the percentage of live spermatozoa varied considerably in different seasons. Table 2 shows that for RShC the season and month of obtaining semen have significant effect ( $P < 0.05$ ) on the motility of spermatozoa, but they do not affect the ejaculate concentration. According to Nickolov and Ivanova (1995), the air temperature as a whole has a stronger impact on the quantity of ejaculates than on the quality indicators of the semen.

In a study of the reproductive capability of cows of the RShC breed, we (Malinova and Nikolov, 2012) have found a marked seasonality of calving, as in the period from January to May 76% of the controlled cows has calved. Calving was most intense during the period January – March. The data on cow reproduction correspond to the results obtained by us of the dynamics of semen production by months. In the period from February to May, an increase in semen concentration and its retention in high levels followed by a sharp decline in June was observed (Fig. 2).

Similar is also the dynamics of the motile spermatozoa percentage (Fig. 3).

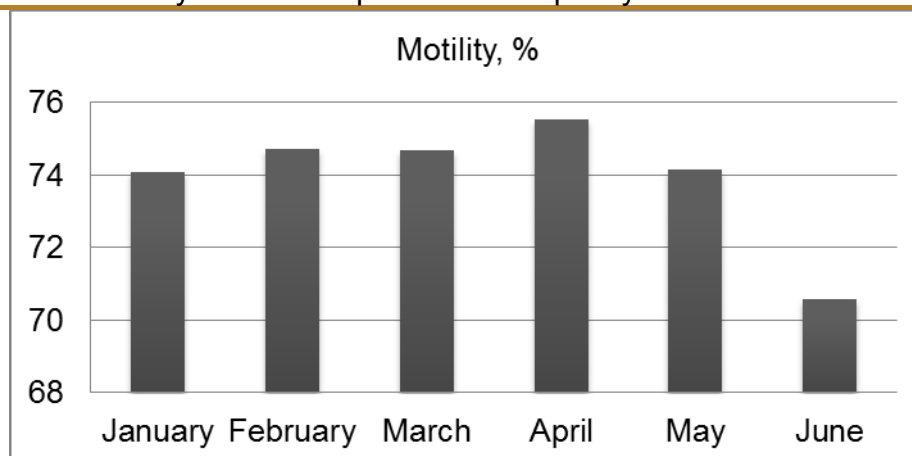


Fig.3 monthly dynamics of sperm motility (%)

In the period from January to April the motility increases and after that decreases. As with concentration, here is also a dramatic decline in June. The causes should rather be sought in the biological characteristics of the breed than in the influence of summer temperatures. In a study of the monthly dynamics of the reproductive capability of bulls of the Holstein and Brown Swiss cattle breeds, Nickolov and Ivanova (1995) have not established a depressing role of summer temperatures on semen quality, although the environmental conditions for these breeds in our country are more unusual than for the RShC breed. On the contrary, during the period from May to August, the reproductive capability of bulls was the highest.

## Conclusions

Bulls of the autochthonous Bulgarian Rhodope Shorthorn breed are characterized by a high reproductive capability. During the first 6 months from the beginning of the exploitation, with age of the bulls from 18 to 24 months, the ejaculate volume was on average of (LS)  $1.74 \pm 0.09$  ml, semen motility was  $74.3 \pm 0.42$  and the concentration was  $1268 \pm 13.1 \times 10^6$  / ml.

There is a significant influence of bull ( $P < 0.001$ ) on reproduction capabilities, but the individual differences in basic characteristics are not high - spermatozoa motility - 71.8-77.0%, semen concentration -  $1222-1324 \times 10^6$ /ml.

During the studied period from January to June, the highest reproductive capability of bulls was during the period from February to April, then decline followed.

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